

Flow cytometry and growth-based analysis of the effects of fruit sanitation on the physiology of *Escherichia coli* in orange juice

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Supplemental Information for

Flow cytometry and growth-based analysis of the effects of fruit sanitation on the physiology of *Escherichia coli* in orange juice

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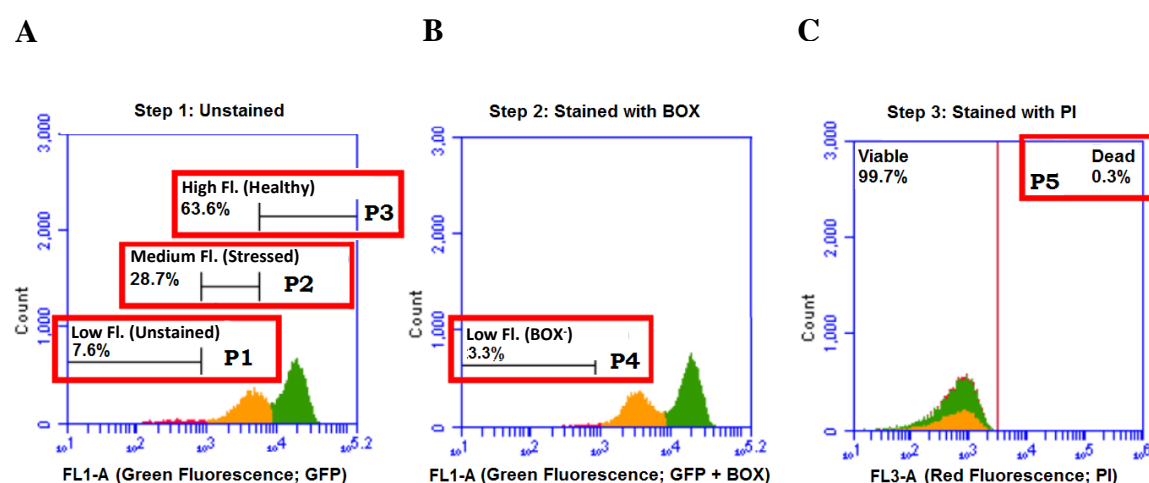
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Supplemental Figure S1



Physiological state	Fluorescence characteristics	Population above
Viable healthy	GFP ⁺ PI ⁻	P3
Viable stressed	Medium GFP	P2
Viable injured	GFP ⁻ BOX ⁺ PI ⁻	(P1 - P4) - P5
Viable low GFP	GFP ⁻ BOX ⁻ PI ⁻	P4
Dead	PI ⁺	P5

Supplemental Figure S1: Simultaneous use of two green fluorophores of GFP and BOX along with red fluorescent viability dye of PI in order to study the number of healthy, stressed, injured and dead *E. coli* K-12 SCC1 cells in OJ. The method of experiment was similar to what was described in Figure 1, with the difference of using BOX in addition to PI. **(A)** Inoculation of cells in OJ resulted in an increase in the number of low GFP and/or GFP⁻ cells. Reduction in GFP fluorescence was presumed to be due to change in internal pH and subsequent denaturation of GFP. Therefore, GFP⁺ were considered healthy whereas low GFP cells were considered to be stressed or injured cells. **(B)** Samples were then stained with BOX in order to determine the percentage of injured cells without a membrane potential. Addition of BOX caused a reduction in the number of cells with low green fluorescence (compared to histogram A), indicating the staining of the injured GFP⁻ cells with BOX. **(C)** Staining the cells with PI showed the percentage of dead cells. Consequently, it was possible to calculate the percentage of injured cells by subtracting the number of dead cells (histogram C) and viable stressed GFP⁻ cells (histogram B) from the pre- BOX staining percentage.